JP 9-194374 A

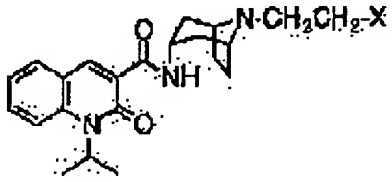
Title of the invention

A therapeutic agent for digestive organ disease

Abstract

Object: To provide a novel therapeutic agent for digestive organ disease having stimulating actions on serotonin 4 receptors.

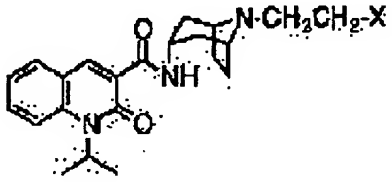
Constitution: A therapeutic agent for digestive organ disease comprising a compound of the formula or its pharmaceutically acceptable salt as an active ingredient.



wherein, X is hydroxymethyl, methoxy, ethoxy or morpholino.

Claims

1. A therapeutic agent for diseases in the digestive organ comprising a compound of formula (I) or its pharmaceutically acceptable salt as an active ingredient.



(I)

wherein, X is hydroxymethyl, methoxy, ethoxy or morpholino.

2. A therapeutic agent for diseases in the digestive organ comprising a compound of formula (II) or its pharmaceutically acceptable salt as an active ingredient.

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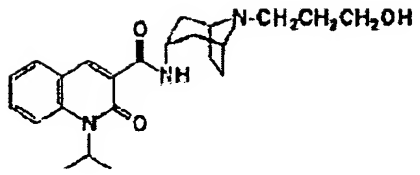

(II)

Detailed description of the invention

Technical field

The present invention relates to a therapeutic agent for digestive organ disease, more specifically an agent useful for improving digestive tract motion based on its stimulating actions on serotonin 4 receptors.

Background Art

Serotonin is a neurotansmitter, which exists in living body, and has a lot of physiological activities. The existence of serotonin 4 receptors has been reported by Dumuis A in 1988 (Molecular Pharmacology, Vol. 34, pp. 880, 1988), in addition to three subtypes of serotonin 1, serotonin 2 and serotonin 3, which had been identified.

Serotonin 4 receptors stimulate the activity of adenylatecyclase together with guanine nucleotide binding protein. It has been implicated that the receptors exist the front of a synapse in nerves and block K channel in cyclic AMP-dependent manner, whereby accelerate the release of acetylcholine.

The receptors are located mainly in central nervous system such as corpus striatum, hippocampus, substantia nigra, but little in cerebral cortex. In addition, it has been reported that the receptors are involved in relaxation of smooth muscle and cardiovascular system in humans and pigs.

A wide variety of activities have been observed in digestive tract. It has been reported that the receptors induce contraction in Guinea pig ileum and proximal colon by way of cholinergic nerves, accelerate contraction of guinea pig ileum by an electric stimulation, and induce Cl release in rat distal colon.

These results indicate that serotonin 4 receptors in digestive tract are involved in

induction and maintenance of peristalsis of digestive tract, and that serotonin 4 receptor stimulants serve to treat and improve digestive tract disease caused by dysperistalsis, by reviving hypoperistalsis of digestive tract.

In fact, it has been known that serotonin 4 receptor stimulants such as cisapride and lenzapride are useful for treating chronic gastritis, diabetes, postoperative gastric motion, heart burn caused by gastric emptying dysfunction, anorexia, abdominal distention, regurgitant esophagitis, intestinal obstruction, constipation and the like by accelerating gastrointestinal motion (Alimentary Pharmacology and Therapeutics, Vol. 6, pp. 273, 1992).

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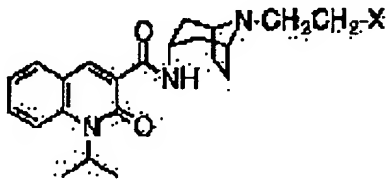
Problem to be solved

The object of the invention is to provide a novel therapeutic agent for digestive organ disease having stimulating actions on serotonin 4 receptors.

Solution

The present inventors have intensively studied to find that a specific quinoline derivative has excellent stimulating actions on serotonin 4 receptors, and accomplished the present invention.

The present invention relates to a therapeutic agent for digestive organ disease comprising a compound of formula (I) or its pharmaceutically acceptable salt as an active ingredient.

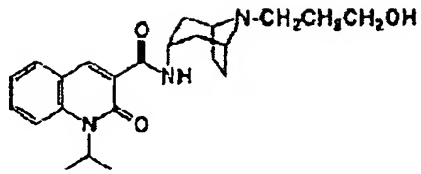


(I)

wherein, X is hydroxymethyl, methoxy, ethoxy or morpholino.

In particular, a preferred compound as the therapeutic agent for digestive organ disease according to the invention, is a compound of formula (II) or its pharmaceutically

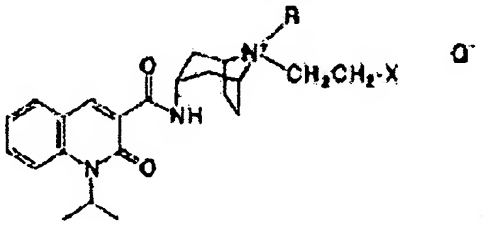
acceptable salt.



(II)

A pharmaceutically acceptable salt of the compound, an active ingredient of the present invention, refers to those salts of inorganic acids such as hydrochloric acid, hydrogen bromide, sulfuric acid, nitric acid, phosphoric acid, and of organic acids such as tartaric acid, maleic acid, succinic acid, formic acid, p-toluenesulfonic acid, benzenesulfonic acid, methanesulfonic acid.

Further, a pharmaceutically acceptable salt includes quaternary salt derivatives of the compound represented by formula (IV), which can be prepared from reaction of a compound of formula (I) with a compound of formula R-Q (III) (wherein, R is lower alkyl, Q is halogen, tosylate or mesylate)

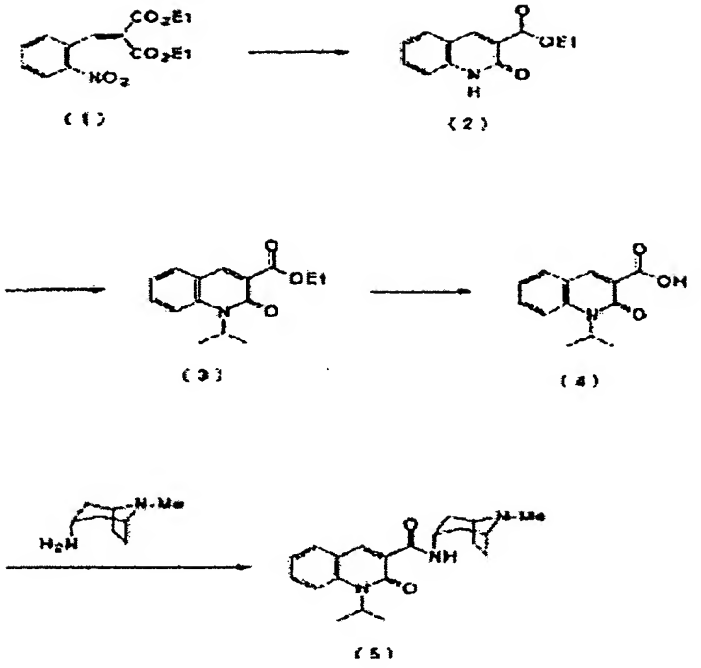


(IV)

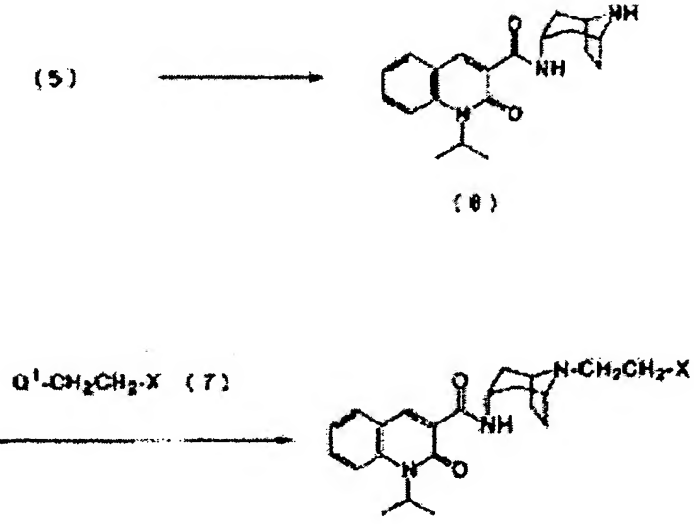
wherein, X, R and Q are as defined above.

The compound as an active ingredient of the present invention can be obtained, for example, by Reaction scheme 1 and Reaction scheme 2, as shown below.

(Reaction scheme 1)



(Reaction scheme 2)



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Embodiments of the invention

The compound as an active ingredient of the present invention may be generally dosed for an adult in the range of 0.01 ~50 mg per day for oral administration and 0.001 ~10

mg per day for intravenous administration, although the amount will vary with the condition of the patient. The doses may be presented in a single dose or as divided doses.

The therapeutic agent for digestive organ disease according to the present invention can be employed in the form of solid preparations such as tablets, pills, capsules, granules, or injections, liquids, emulsions, suppository and the like.

In manufacturing solid preparations, conventional additive agents can be used, for example, excipients, disintegrators, binding agent, lubricant, coating material. The granulation methods using stirring, fluidizing bed and milling may be employed.

The compounds according to the present invention act on serotonin 4 receptors and exhibit serotonin-like stimulating activity. Therefore, the compounds according to the present invention are useful for treating chronic gastritis, diabetes, postoperative gastric motion, heart burn caused by gastric emptying dysfunction, anorexia, abdominal distention, regurgitant esophagitis, intestinal obstruction, constipation and the like.

The invention will be more fully described by way of the following preparation, reference and working examples, and assays.

Preparation 1

Preparation of endo-N-[8-(3-hydroxypropyl)-8-azabicyclo[3.2.1]oct-3-yl]-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide

(1) Endo-N-(8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide hydrochloride

A solution of endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide 4.0 g and 1-chloroethyl chloroformate 1.24 ml in 1,2-dichloroethane 50 ml was refluxed under heat for 1 h. After solvent was removed by distillation under reduced pressure, methanol 40 ml was added and stirred under heat for 1 h. The solvent was removed by distillation, and resultant residues were chromatographed on silica gel (chloroform : NH_3 saturated methanol 20 : 1). A recrystallization from ethyl acetate gave the titled compound 2.2 g. mp; > 270℃

(2) Endo-N-[8-(3-hydroxypropyl)-8-azabicyclo[3.2.1]oct-3-yl]-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide

A solution of endo-N-(8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide 2.0 g, 3-bromo propanol 0.53 ml and potassium carbonate 0.81 g in ethanol 50 ml was stirred at room temperature for 10 h. This was poured into water and extracted with chloroform. The chloroform layer was washed with water and dried over sodium sulfate. The solvent was removed by distillation to yield residues, which were chromatographed on silica gel (chloroform : methanol 20 : 1). A recrystallization from ethyl acetate gave the titled compound (test compound 1) 0.51 g.

mp; 171-172℃ (ethyl acetate)

Preparation 2

Preparation 1 was likewise conducted to obtain endo-N-(8-(2-methoxyethyl)-8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide hydrochloride (test compound 2), using 2-methoxyethyl bromide instead of 3-bromo propanol in Preparation 1 (2).

mp; 247-249℃ (ethyl acetate)

Preparation 3

Preparation 1 was likewise conducted to obtain endo-N-(8-(2-ethoxyethyl)-8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide (test compound 3), using 2-ethoxyethyl bromide instead of 3-bromo propanol in Preparation 1 (2).

mp; 99-100℃ (isopropyl ether)

Preparation 4

Preparation 1 was likewise conducted to obtain endo-N-(8-(2-morpholinoethyl)-8-azabicyclo[3.2.1]oct-3-yl)-1-isopropyl-2-oxo-1,2-dihydro-3-quinolinecarboxamide (test

compound 4), using 2-morpholinoethyl bromide instead of 3-bromo propanol in Preparation 1 (2).

mp; 177-178℃ (ethyl acetate-isopropyl ether)

("omitted")

Working Example 1

Formulation (for 1 tablet)

The compound of Preparation 1 (test compound 1)	10 mg
lactose	50 mg
corn starch	59.75 mg
crystalline cellulose	27 mg
carmellose calcium	27 mg
hydroxypropyl cellulose	5.25 mg
magnesium stearate	1 mg
total	180 mg

The compound of Preparation 1, lactose, corn starch, crystalline cellulose, carmellose calcium were mixed homogeneously and 10% aqueous hydroxypropyl cellulose solution was added to the mixture. This was kneaded, dried and passed through 30M sieve to form uniform granules. Magnesium stearate was added to prepare a tablet.

Working Example 2

Formulation (for 1 tablet)

The compound of Preparation 1 (test compound 1)	10 mg
lactose	50 mg
corn starch	59.75 mg
crystalline cellulose	27 mg
carmellose calcium	27 mg

hydroxypropyl cellulose	5.25 mg
magnesium stearate	1 mg
total	180 mg

The compound of Preparation 1, lactose, corn starch, crystalline cellulose, carmellose calcium were mixed homogeneously and 10% aqueous hydroxypropyl cellulose solution was added to the mixture. This was kneaded, dried and passed through 30M sieve to form uniform granules. Magnesium stearate was added to prepare a tablet.

Working Example 3

Formulation (for 1 tablet)

The compound of Preparation 1 (test compound 1)	1 mg
lactose	59 mg
corn starch	59.75 mg
crystalline cellulose	27 mg
carmellose calcium	27 mg
hydroxypropyl cellulose	5.25 mg
magnesium stearate	1 mg
total	180 mg

The compound of Preparation 1, lactose, corn starch, crystalline cellulose, carmellose calcium were mixed homogeneously and 10% aqueous hydroxypropyl cellulose solution was added to the mixture. This was kneaded, dried and passed through 30M sieve to form uniform granules. Magnesium stearate was added to prepare a tablet.

Working Example 4

Formulation (for 1 tablet)

The compound of Preparation 1 (test compound 1)	0.1 mg
lactose	59.9 mg
corn starch	59.75 mg

crystalline cellulose	27 mg
carmellose calcium	27 mg
hydroxypropyl cellulose	5.25 mg
magnesium stearate	1 mg
total	180 mg

The compound of Preparation 1, lactose, corn starch, crystalline cellulose, carmellose calcium were mixed homogeneously and 10% aqueous hydroxypropyl cellulose solution was added to the mixture. This was kneaded, dried and passed through 30M sieve to form uniform granules. Magnesium stearate was added to prepare a tablet.

Assay 1, Stimulating actions on serotonin 4 (5-HT4) receptors

§ Animals: Male Hartley guinea pig (250~400 g)

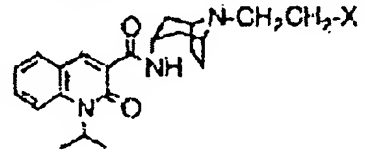
§ Methods: Longitudinal muscle, which was obtained from ileum 10-20 cm away from ileocecum of Hartley guinea pig, was used in experiment. Longitudinal muscle prep was suspended in Krebs solution (32~34℃) and 95% O_2, 5% CO_2 were ventilated through about 0.8 g load. Reactions were observed in equivalent intervals. An electrical stimulation of 1 millisecond was applied in the frequency of 0.2 Hz for 2 ~ 3 hr to stabilize. Voltage was lowered to stabilize for further 1 hr. After ensuring that electric stimulation -induced contraction was enhanced at 10^{-8}M 5-HT, activities of test compounds was determined. The test compounds were cumulatively added after at least 45 minutes repose of prep.

<References> Craig, D. A. and Clarke D. E.: Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine 4 receptor: The Journal of Pharmacology and Experimental Therapeutics 252:1378-1386, 1990 .

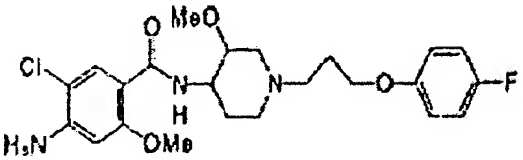
§ Test Samples: Test compounds and cisapride were dissolved in distilled water or DMSO to dilute. Samples were prepared such that the concentration of DMSO in bath is below 0.3% for use in experiment.

The structures of each test sample are provided in Table 1 below:

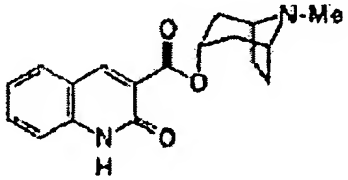
[Table 1]

formula 	
test compound	X
1	CH_2OH
2	OMe
3	OEt
4	

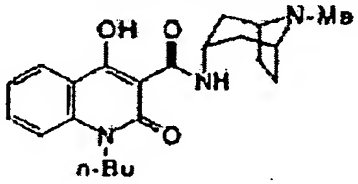
Comparison compound 1: cisapride



Comparison compound 2: compound disclosed in US patent No. 5,106,851.



Comparison compound 3: compound disclosed in EP 0 458 636 A1.



§ Production of results: The greatest contraction by test samples was considered as 100% and the concentration (EC50) at which enhancement of contraction by an electric stimulation is 50% was determined.

Analytical method: RS1 (BBN software program) was used.

§ Results: Results are listed in Table 2.

[Table 2]

Test Compound	EC50 (nM)
1	32.0
2	15.0
3	18.7
4	25.5
Comparison Compound	EC50 (nM)
1	271.3
2	>3000
3	>3000

Assay 2, 5-HT4 receptor binding inhibition activity

§ Methods

1. Preparation of membrane sample (5-HT4 receptors)

1) Corpus striatum of guinea pig

Corpus striatum was taken from guinea pig (Hartley, Male, Charles river), homogenized in 20 times amount of 50 mM HEPES buffer (pH 7.4) and centrifuged at 48,000 G (27,000 rpm) for 10 min. Pellet was resuspended and incubated at 37℃ for 30 min. This was then centrifuged at 27,000 rpm for 10 min and pellet was suspended in HEPES buffer containing 10^{-6}M pargyline and 0.1% ascorbic acid and used for binding assay.

2) Ileal longitudinal muscle of guinea pig

Ileal longitudinal muscle of guinea pig was homogenized with Teflon glass homogenizer in 0.32M sucrose and centrifuged at 900 G for 10 min. Upper lipid layer and pellet were removed and supernatant was centrifuged at 100,000 G (48,000 rpm) for 1 hr. Pellet was resuspended in 50 mM HEPES buffer and incubated at 37℃ for 30 min, and then centrifuged at 48,000 rpm for 20 min. Pellet was suspended in HEPES buffer containing 10^{-6}M pargyline and 0.1% ascorbic acid and used for binding assay.

2. 5-HT4 receptor binding inhibition assay

Membrane sample was incubated with [^{3}H]GR113808 (0.1 nM) (Amersham) and test compounds at final volume of 1.0 ml at 25℃ for 30 min. Binding in the presence of 5-HT (3 x 10^{-5}M) was considered as non-specific binding. B/F separation was performed on harvester with GF/B filter, which was treated with 0.1% polyethyleneimine, and rinsing was conducted once.

§ Test samples: Test samples were dissolved in DMSO and tested at final concentration of 1% DMSO.

§ Production of results: In binding inhibition assay, IC50 value of a drug was determined according to IC50 program of windows-origin (Microcal software).

§ Results: Results are listed in Table 3.

[Table 3]

Test Compound	Ileum (n = 3) IC50 (nM)
1	42.19 ± 6.29
2	78.50 ± 14.63
3	68.70 ± 7.32
4	78.14 ± 14.52
cisapride	97.47 ± 9.17

Assay 3, Receptor Selectivity

§ Methods

1) D2 receptors

Affinity to D2 receptors was determined from inhibitory activity of binding of [^{3}H] raclopride to rat corpus striatum membrane. Rat corpus striatum was homogenized in 50 mM Tris HCl buffer (pH 7.4) and centrifuged at 48,000 G. Pellet was washed once with Tris HCl buffer. Pellet was suspended in 50 mM Tris HCl buffer (containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) and used as membrane

sample. Membrane sample (0.5 mg protein/ml) was reacted with 1 nM [^{3}H] raclopride at 25℃ for 60 min.

At the end of reaction, membrane was captured with harvester. Binding in the presence of 10 μM haloperidol was considered as non-specific binding.

2) 5-HT3 receptors

Affinity to 5-HT3 receptors was determined from inhibitory activity of binding of [^{3}H] GR65630 to rat cerebral cortex membrane. Rat cerebral cortex was homogenized in 50 mM HEPES buffer (pH 7.4) and centrifuged at 48,000 G. Pellet was washed once with HEPES buffer. Pellet was suspended in 50 mM HEPES buffer and used as membrane sample. Membrane sample was reacted with 0.2 nM [^{3}H] GR65630 at 37℃ for 30 min.

At the end of reaction, membrane was captured with harvester. Binding in the presence of 1 μM zacopride was considered as non-specific binding.

3) 5-HT1A receptors

Affinity to 5-HT1A receptors was determined from inhibitory activity of binding of [^{3}H] 8-OH-DPAT to guinea pig cerebral cortex membrane. Guinea pig cerebral cortex was homogenized in 50 mM Tris HCl buffer (pH 7.7) and centrifuged at 48,000 G. Pellet was washed once with Tris HCl buffer. Pellet was suspended in 50 mM Tris HCl buffer (containing 0.01 mM pargyline, 0.1% AsH_2, pH 7.7) and used as membrane sample. Membrane sample was reacted with 1 nM [^{3}H] 8-OH-DPAT at 37℃ for 15 min.

At the end of reaction, membrane was captured with harvester. Binding in the presence of 10 μM 5-HT was considered as non-specific binding.

<Reference> GROSSMAN, C. J., KILPATRICK, G. J. & BUNCE, K. T. (1993) Development of aradioligand binding assay for 5-HT4 receptors in guinea-pig and rat brain. Br. J. Pharmacol., 109, 618-624.

§ Test samples: Test samples were dissolved in DMSO and tested at final concentration

of 1% DMSO.

§ Production of results: In binding inhibition assay, IC50 value of a drug was determined according to IC50 program of windows-origin.

§ Results

[Table 4]

Test Compound	1	2	3	4	cisapride
Dopamine D2 receptor antagonizing activity (IC50, nM)	-	-	-	-	107.5
5-HT3 receptor antagonizing activity (IC50, nM)	977	811	423	609	97.7
5-HT1A receptor antagonizing activity (IC50, nM)	-	-	-	-	64.7

"-" indicates that no action was observed at 10 μM.

Assay 4, Digestive Tract Motion-Stimulating Activity (dogs, after meal)

§ Animals: Female Beagles

§ Methods: Dog models was prepared and used according to methods of Yoshida et al.

Postranduser (sensor for measuring contracting force; F-121S manufactured by Starmedical) was attached to gastric vestibular region (3 cm from pyloric limbus toward mouth), duodenum (5 cm from pyloric limbus toward anus), jejunum (70 cm from pyloric limbus toward anus), end of ileum (5 cm from ileocolic junction toward mouth), and colon (5 cm from ileocolic junction toward anus), after anesthesia with pentobarbital (30 mg/kg, i.v.) and celiotomy. Lead wire of Postranduser was drawn out of back through hypoderm from flank to link connector. After surgery, dogs were put on protection jacket such that connector was placed therein. Onward 2 weeks after surgery, digestive organ motility was measured. In order to measure freely, contractive

motion was observed in individual digestive organ using telemeter (an electric wave-type data transmitter; DAT-80T manufactured by Starmedical), which was linked to connector. Data was stored in a computer (PC9801FA manufactured by NEC) through telemeter (a recorder; DAT-80A manufactured by Starmedical) for presevation and analysis.

Drugs (vehicle, cisapride and test compound 1) were administered intravenously from propodite on 2 weeks after feeding meals (1116 kcal; manufactured by Oriental yeast).

<Reference> Yoshida. N. and Ito. T.: AS-4370, a new gastrokinetic agent, enhances upper gastrointestinal motor activity in conscious dogs: The Journal of Pharmacology and Experimental Therapeutics, 257, 781-787, 1991.

§ Test Samples: Cisapride and test compound 1 were dissolved in 0.5% dl-lactic acid solution. 0.5% dl-lactic acid solution was used as vehicle.

§ Production of results: Digestive organ motion was produced by a computer every 15 min as motility index (M. I.) (g·min). The 15 min average value of stimulating activity for 0~1 hr after administration (i.e., total M. I. (%) for 0~1 hr/4) was calculated for gastric vestibular region, duodenum and jejunum, while the 15 min average value of stimulating activity for 0~0.5 hr after administration (i.e., total M. I. (%) for 0~0.5 hr/2) was calculated for ileum and colon. Further, the 15 min average value for 30 min before administration was considered as 100% M. I.

§ Results: Results are reported in Figure 1.

Assay 5, Toxicity test of oral administration continued for 14 days in rats

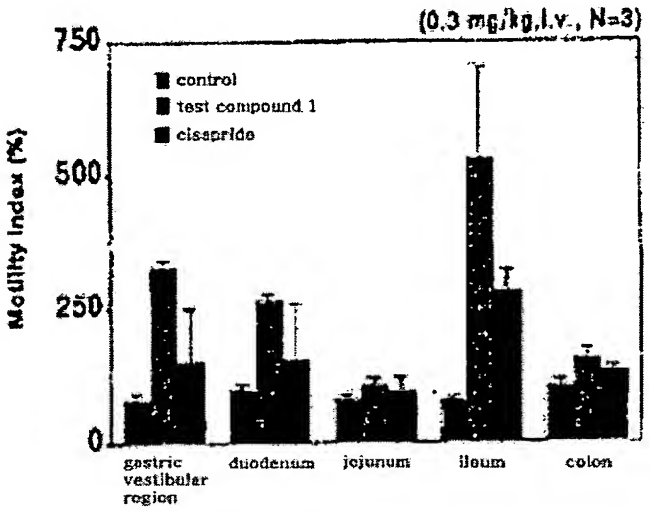
Wistar male and female rats (7 rats per group) aged 5 weeks were used for toxicity test when test compound 1 of Preparation 1 was dosed orally at 75, 150 and 300 mg/kg once a day for 14 days. During dosing period, general condition was observed and body weight was measured. At the end of dosing, blood and urine test, organ weighing and pathological diagnosis were conducted.

As a result, at a dose of 300 mg/kg, reduction and inhibition of increase in body weight were observed and two male rats exhibited tremor and convulsion and died. The

increase in the activity of transamylase was admitted for female at beyond 150 mg/kg and for male at 300 mg/kg. Non-toxic dose was regarded as 75 mg/kg and toxicity was considered week taking into consideration of effective doses.

Brief description of figure

Figure 1. A graph showing the effect of quinoline carboxylic acid compound of the present invention on digestive tract motion in dogs after meal.



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